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Effect of Different Temperature Regimes on the Germination of *Pseudolysimachion pusanensis* (Y. N. Lee) Y. N. Lee Seeds

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Abstract: In this study, we determined the germination response in the seeds of the rare plant *Pseudolysimachion pusanensis* (Y. N. Lee) Y. N. Lee to different temperatures. *P. pusanensis* seeds were collected from the Baekdudaegan National Arboretum, South Korea, in November 2019, and dried. Dry seeds were placed at constant and alternating temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C) to determine their germination percentage (GP). The seeds were exposed to 59 temperature combinations ranging from 5 °C to 43 °C using a thermal gradient plate. The photoperiod was set at 12:12 h (light:dark) and germination assays were performed five times a week. Subsequently, the seed GP and the number of days required to reach 50% of the germination (T_{50}) were determined. The highest final GP was 94.38%, with a T_{50} value of 9.26 d at 15 °C. However, the mean germination time was 12.5 d at 15 °C, and linear regression using $1/T_{50}$ revealed that the base temperature ranged from 2.69 °C to 4.68 °C. These results for *P. pusanensis* seeds stored in a seed bank provide useful data for the native plants horticulture industry and can also be utilized for storage management.

Keywords: seed germination; thermal gradient plate; seedbank; *Pseudolysimachion pusanensis*; base temperature; storage management

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1. Introduction

Species in the genus *Veronica* represent a large group of annual or perennial herbs [1] that are distributed across Eurasia in the northern hemisphere through to the southern hemisphere (Australia, New Zealand, New Guinea) [2,3]. The *Veronica* subgenus *Pseudolysimachium* comprises approximately 450 species and is used for ornamental horticulture in gardening, including *V. spicata* and *V. longifolia* [4,5]. This subgenus is particularly popular in horticulture because the flowers bloom from spring to autumn, resulting in a long flowering period. Moreover, these species are easy to manage when planted in a garden. In addition, this subgenus has gained attention in recent years due to the ease of hybridization, and the addition of various flower colors and new cultivars [6].

Veronica pusanensis Y. N. Lee [7] was first identified in Gijang-gun, Busan, Republic of Korea, and exhibits several morphological differences compared to other species of the genus. Therefore, Y. N. Lee renamed *V. pusanensis* as *Pseudolysimachion pusanensis* (Y.'N. Lee). Y. N. Lee [8]. *P. pusanensis* is a rare plant that is classified as data-deficient [9] and is distributed only in Busan [10]. *P. pusanensis* is characterized by purple racemes and is of dwarf type, and grows by crawling along the ground. To maintain *P. pusanensis* horticulturally with different traits and to conserve the genetic resources of this rare plant, it is important to investigate its optimal conditions for growth.

In Korea, basic growth research has been carried out for enhancing the development of new ornamental plants and cultivating excellent varieties of 20 plant species in the genus *Veronica* [11] in order to understand the flowering and light conditions of the genus [12], along with plant propagation research through stem cutting using various

types of auxins [13]. Although this research is actively conducted to secure the breeding material of native genera, the primary method to protect and preserve original species remains seed storage. Internationally, seeds are stored in seed vaults and seed banks. In 2008, the Svalbard Global Seed Vault in the frozen Norwegian island of Spitsbergen was commissioned for the storage of crop seeds, and the Baekdudaegan Global Seed Vault (BGSV) was instituted at the Baekdudaegan National Arboretum to store the seeds of wild plants [14]. Seed bank collections of wild species contribute to habitat restoration and species reintroductions [15–17]. Therefore, further investigations on seed germination and seedling growth are necessary to effectively use the seeds of wild species and maintain the processes in seed banks.

Additionally, wild plant seeds show different types of dormancy and dormancy depths [18]. Dormancy is a crucial plant trait to prevent germination during unfavorable conditions, and serves as insurance for seed survival [19,20]. Among the genus *Veronica*, *V. parnkalliana* seeds show morphophysiological dormancy (MPD) due to undeveloped embryos and the inhibition of the physiological mechanism in the embryo [18,21], and *V. kiusiana* seeds show non-deep simple MPD according to the physiological dormancy depth [10]. Conversely, *V. pusanensis*, *V.'dahurica*, *V. rotunda*, *V. nakaiana*, *V. pyrethrina*, and *V. kiusiana* show morphological dormancy (MD) due to undeveloped embryos [10].

The base temperature refers to the basic temperature requirements of herbaceous species for seed germination and emergence. The estimation of the base temperature can help to predict seed germination and determine the suitable sowing time based on the local temperature [22]. Germination processes are regulated by the accumulated temperature above the base temperature [23]. Further, the seed germination index indicates the dynamics of the germination process, allowing for a comparison of species with similar germination [24].

With the aim of contributing to the horticulture industry using native plants, in this study, we investigated the most basic morphology of *P. pusanensis* seeds and further estimated the base temperature, according to the germination index, for the germination of dry *P. pusanensis* seeds.

2. Materials and Methods

2.1. Material Collection and Seed Processing

P. pusanensis seeds were collected from the exhibition garden of the Baekdudaegan National Arboretum (Bonghwa-gun, 37°00′31.3″ N 128°49′49.0″ E). In Bonghwa-gun, the average annual temperature ranges from −3 °C to 22.5 °C and the annual rainfall is >1229 mm.

P. pusanensis plants exhibit indeterminate inflorescence; therefore, they produce differentially matured seeds. The basal flowers of the inflorescence used in this study bloomed at the end of August, whereas the uppermost flowers bloomed in mid-October. Plants, with seeds just before their release from the inflorescence, and free from insects, were collected on 20 November 2019. Subsequently, they were dried under the tree canopy, and then were passed through various sieves and winnowed to extricate the filled seeds. Filled seeds were then dried in a drying room [temperature: 15 °C; relative humidity (RH): 15%] and sealed in aluminum packages for storage in a short-term storage room (temperature: 4 °C; RH: 15%) in December 2019 until further experiments. During the experiments, the seeds were stored with silica gel over the short term.

2.2. Seed Morphology

Ten seeds and a seedling were photographed using a digital microscope (Leica Microsystems DVM6-Leica PlanApo; Leica, Wetzlar, Germany) to determine their morphology. Seed size was measured from digital images captured using the DVM6 microscope equipped with the Leica Application Suite X (LAS X) software. Seed images were captured, and the seed length, width, and cross-sectional area, along with the length of the embryo at dispersal and just before germination, were measured using the digital microscope and

associated software. Subsequently, embryo:seed (E:S) ratios at seed dispersal and just before germination were assessed using paired *t*-tests.

2.3. Estimation of 1000-Seed Weight

Ten replicates with 100 seeds were weighed and used to calculate the mean 1000-seed weight.

2.4. Estimation of Initial Seed Moisture Content (MC)

Seed MC (%, fresh-weight basis) was determined before storage in sealed containers at the Baekdudaegan seed bank. In the drying room, seeds were equilibrated for 1–2 weeks to reach the equilibrium point (RH: 15%) to measure their equilibrium relative humidity (ERH) using a Rotronic hygrometer (HC2-AW; Rotronic Instruments UK Ltd., Crawley, UK) connected to HW4-E software V3.9.0 [25]. Seed MC was then determined using the low-temperature oven drying method, as detailed by the International Seed Testing Association (ISTA) [26].

Briefly, samples were ground and placed on aluminum foil dishes, with each ground seed weighing 1 g. Seeds were dried at 103 ± 2 °C for 17 ± 1 h [26] and cooled in a desiccator. After cooling, seeds were weighed to determine weight loss. All samples were assessed in quadruplicates.

Seed MC was calculated using the following formula and expressed as a percentage of the fresh seed weight:

$$\text{MC} = [(\text{weight of fresh seeds} - \text{weight of dried seeds})/\text{weight of fresh seeds}] \times 100 \quad (1)$$

2.5. Estimation of Total Protein Content

Seeds were ground and their protein content was determined via the standard Kjeldahl method [27] using a Kjeltec analyzer (Kjeltec 8400; FOSS Ltd., Hillerød, Denmark). Protein content estimation was performed in triplicates, and a factor of 6.25 was used with the N content [27]. The values obtained were then averaged.

2.6. Total Crude Fat Extraction Using the Soxhlet Method

Total crude fat was extracted from the seeds using a Soxhlet extractor, which was developed by Franz von Soxhlet in 1879 for the extraction of lipids from a solid material (seeds) to determine the percentage of crude fat.

Seeds were ground to a particle size of <0.1 mm in liquid nitrogen using a mortar and pestle. Approximately 1 g of crushed seeds were weighed and transferred to a 28 mm × 100 mm cellulose thimble (No. 84; Advante Corp., Tokyo, Japan). Subsequently, approximately 100 mL of diethyl ether (boiling point: 60–80 °C) was boiled in a Soxhlet apparatus (100 mL round-bottomed flask) fitted with a condenser, using an electrothermal heater (EAM 9202-06; Tops Misung Scientific Co., Seoul, Korea), and crude fat was extracted under reflux with petroleum ether for 16 h (3–4 cycles/h) according to the International Official Methods of Analysis (AOAC) section 920.39 [27]. The water cooler (HB-207M; Hanbaek Corp., Bucheon, Korea) was maintained at 3–4 °C using a solvent mixture of methanol:water (1:9). Upon completion of extraction, the defatted sample was removed. The distillation flask containing the total crude fat was then oven-dried at 50 °C for 15 min and cooled in a desiccator. The flask and its contents were then weighed.

All extractions were performed in quadruplicates, and the mean crude fat content was calculated using the following formula and expressed as a percentage of seed weight.

$$\text{Total crude fat content (\%)} = [W_3 - W_2/W_1] \times 100 \quad (2)$$

where W_1 is original weight of the sample, W_2 is weight of the empty flask, and W_3 is weight of the flask and fat.

2.7. Germination Test and Index

Germination test under different temperature regimes as described in Section 2.8 were performed on 14 April 2020. Four replicates with ten seeds each per condition were sown on the surface of 1% agar (Sigma-Aldrich, St. Louis, MO, USA) in 60 mm × 15 mm plastic Petri dishes (SPL Life Sciences Co. Ltd., Pocheon, Korea). The number of germinated seeds was counted on weekdays for five weeks. Germinated seeds, with radicle length of 0.5–1 mm, were then observed under the digital microscope. The experiments were terminated when no further germination was observed for over one week.

Final viability of non-germinated seeds was verified using the triphenyltetrazolium chloride (TTC) test. The non-germinated seeds were immersed in a 1% aqueous solution of 2,3,5-triphenyl-2H-tetrazolium chloride at 30 °C overnight (12 h). We identified the viable and nonviable seeds using a microscope; pink- or red-stained embryos were considered viable, whereas unstained embryos were considered nonviable [18].

The germination index was calculated based on the germination percentage (GP), mean germination time (MGT), and 50% of the final germination (T_{50}). GP was calculated using the following formula [26]:

$$GP = (N/S) \times 100, \quad (3)$$

where N is the sum of the number of seeds germinated until the end of the germination test and S is the total number of seeds.

The final germination percentage (FGP) was calculated using the total number of sow seeds, excluding dead seeds (unstained embryos), as the parameter.

MGT was estimated using the following formula [28]:

$$MGT = \Sigma(T_i \times N_i)/N, \quad (4)$$

where T_i is the number of days from the beginning of germination until day T and N_i is the number of seeds germinated on day T.

T_{50} was determined using the formula given below [29]:

$$T_{50} = t_i + [\{(N + 1)/2 - n_i\} \times (t_j - t_i)]/(n_j - n_i), \quad (5)$$

where t_i is time period before reaching 50% of the final germination, n_i is the number of seeds that emerged at t_i, t_j is the time after t_i, and n_j is the number of seeds that emerged at t_j.

2.8. Temperature Regimes

2.8.1. Experiment 1: Seven Constant Temperature Regimes

Each plastic Petri dish was placed in a transparent plastic box and kept in a germination chamber (TGL-1S; Espec Mic Corp., Aichi, Japan) and then in a growth chamber (TGC-130H; Espec Mic Corp., Aichi, Japan) at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C, with 12:12 h (light:dark) cycles for all temperature regimes. The seeds were sown in agar medium and examined for germination, as described in Section 2.7.

2.8.2. Experiment 2: Fifty-Nine Different Temperature Regimes

The base temperature (Tb) was defined using a linear model of development rate against temperature. To determine the base temperature, the seeds were incubated in a microthermal gradient plate (ONSOL Corp., Suwon, Korea) with a 12:12 h (light:dark) photoperiod (white fluorescent light: 40 ± 10 μmol·m^{-2}·s^{-1}). The thermal gradient plate (TGP) was based on 60 temperature combinations of 10 × 6 (horizontal × vertical) with a temperature range of 7–43 °C at 4 °C intervals and 5–30 °C at 5 °C intervals. However, during the evaluation process, an abnormal temperature occurred in one cell, and it was recorded in the data logger. It was excluded because the data were not reliable outside the evaluation temperature range (±1 °C). Therefore, only 59 conditions were used for

data analysis. The Petri dishes were placed in a completely randomized design on the TGP. There are instructions found in 2.7 for agar medium conditions, germination checking, etc.

2.9. Statistical Analysis

Data were analyzed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Statistically significant differences between the variables were determined using one-way analysis of variance with Tukey's multiple range tests assessed at $p < 0.05$. Linear regression plots and graphs were generated using SigmaPlot 12.5 software (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Seed Morphology

P. pusanensis plants exhibit indefinite inflorescences (Figure 1a). The flowers have inferior ovaries (Figure 1b) with ovules inside. When the ovaries mature, their color changes from green to reddish brown, and they become dry and release seeds (Figure 1c,d). The seeds were glossy, flat and round, and 1.05 ± 0.21 mm (length) × 0.96 ± 0.17 mm (width) in size, with light or dark brown testae (Figure 1e,f) and undeveloped embryos at dispersal (Figure 1g). The E:S ratios at dispersal and just before germination were 0.44 ± 0.05 and 0.69 ± 0.08, respectively, indicating an increase of 56.0 ± 2% during germination. After the embryo developed, the radicle emerged through the hilum of the seed (Figure 1h).

3.2. Seed Characteristics

When the seeds reached an ERH of 17.58%, the seed MC and 1000-seed weight were 4.32 ± 0.05% and 0.1188 ± 0.0011 g, respectively. Additionally, the total crude fat and protein contents of the seeds with a FGP ≥ 85% were 22.43 ± 3.32% and 15.94 ± 0.09%, respectively.

3.3. Constant Temperature Regimes

The cumulative germination in *P. pusanensis* seeds was not affected at 35 °C. However, the GP varied from 0.0 to 90.0 ± 7.07% for temperature regimes ranging from 5 °C to 35 °C. Further, the GP increased from 5 °C to 15 °C, but began to decrease with a further increase in temperature (Figure 2). The TTC test was used to determine the number of viable and non-viable seeds, and it reflected the average FGP. The highest FGP (94.38 ± 3.29%) was observed five weeks after sowing at 15 °C.

The germination index of the seeds was determined using the MGT and the inverse of the time required to reach 20%, 50%, and 80% of the FGP ($1/T_{(FGP)}$, d^{-1}). Moreover, $1/T_{(FGP)}$ was plotted as a function of temperature and was analyzed using linear regression. When $1/T_{(GP)} = 0$, we estimated the base temperature (T_b).

P. pusanensis seeds exhibited the fastest response time, with T_{20} = 4.50 days, T_{50} = 5.38 days, and T_{80} = 7.80 days at 25 °C, and inverse values of 0.222, 0.186, and 0.128, respectively (Figure 3). Similar values were obtained for the germination index of MGT. However, at 5 °C and 25 °C, the germination index could not be determined because of a low GP. By contrast, the slowest response was observed at 10 °C (0.0432) in the sub-optimal range. A T_b value of 3.98 ± 0.65 °C was obtained when linear regression plots were fitted with $1/T_{(FGP)}$. These results demonstrated that a constant temperature of 15 °C affected germination in *P. pusanensis*. Furthermore, the initial GP and T_{50} were at 5 days after sowing (data not shown) and 9.2 days, respectively.

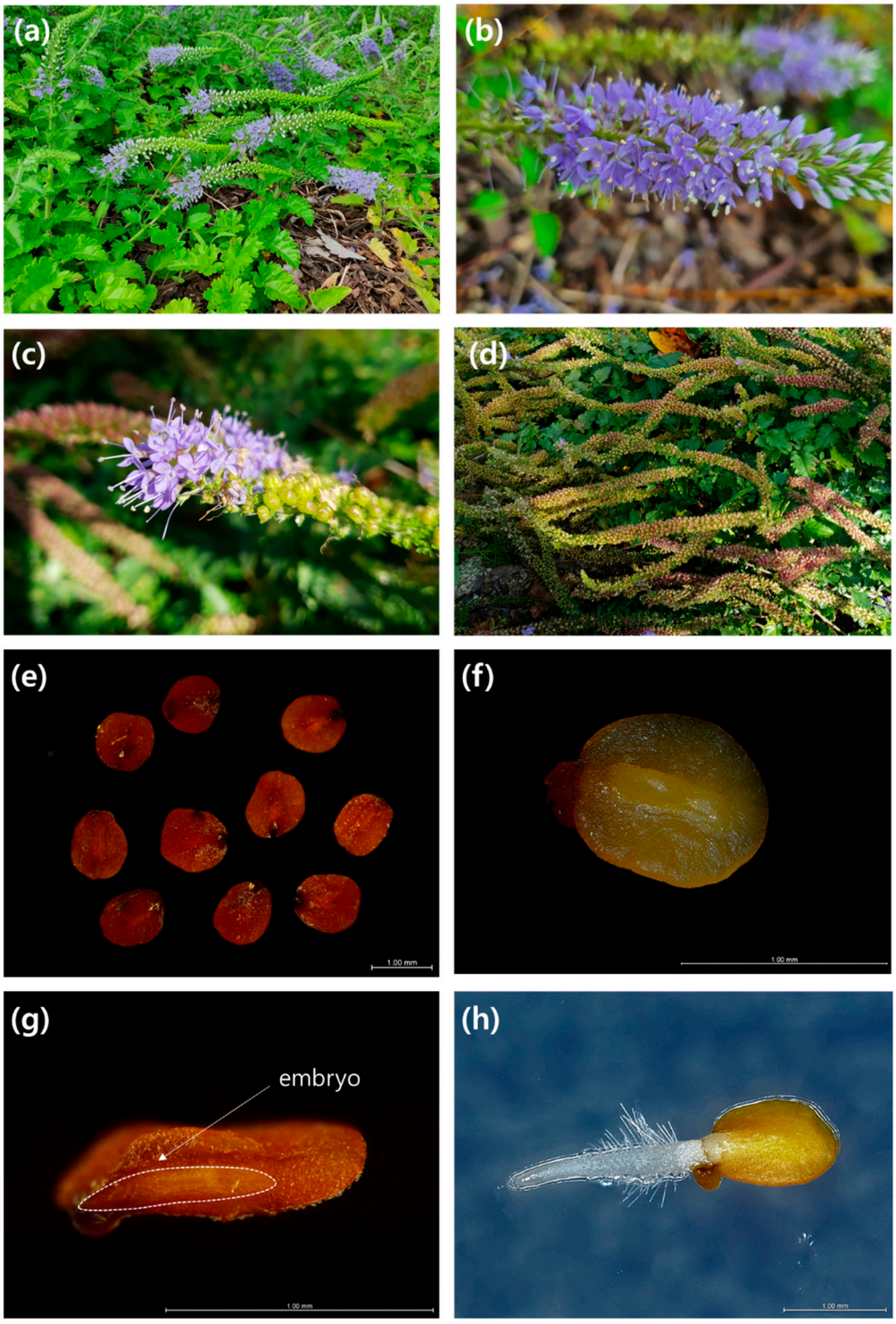


Figure 1. Morphology of *Pseudolysimachion pusanensis* (Y. N. Lee) Y. N. Lee. (**a**) A field of *P. pusanensis* at Bonghwa-gun. (**b**) Flowers on the basal inflorescence bloomed on 3 August, 2019. (**c**) Flowers on the distal inflorescence bloomed on September 30, 2019. (**d**) The seed color in the ovaries varied from green to brown on October 20, 2019. (**e**) Seeds at dispersal. (**f**) Digital image of a seed. Cross-section of a seed showing (**g**) an undeveloped embryo and (**h**) radicle emergence; scale bar = 1 mm (applies to (**e**–**h**)).

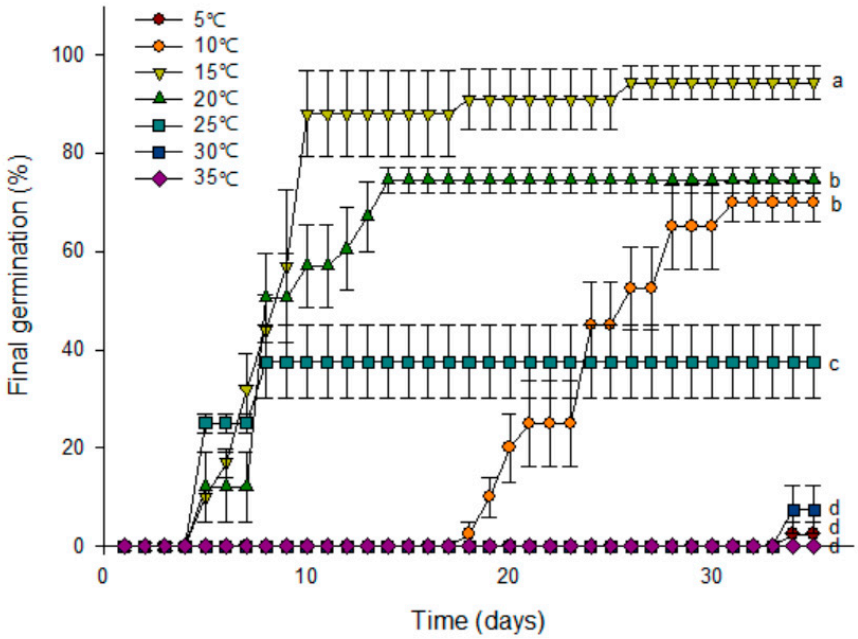


Figure 2. Final germination of *P. pusanensis* seeds at constant temperature regimes ranging from 5 °C to 35 °C. Bars represent mean ± SE (n = 4). Same lowercase letters above columns indicate no significant difference ($p < 0.001$).

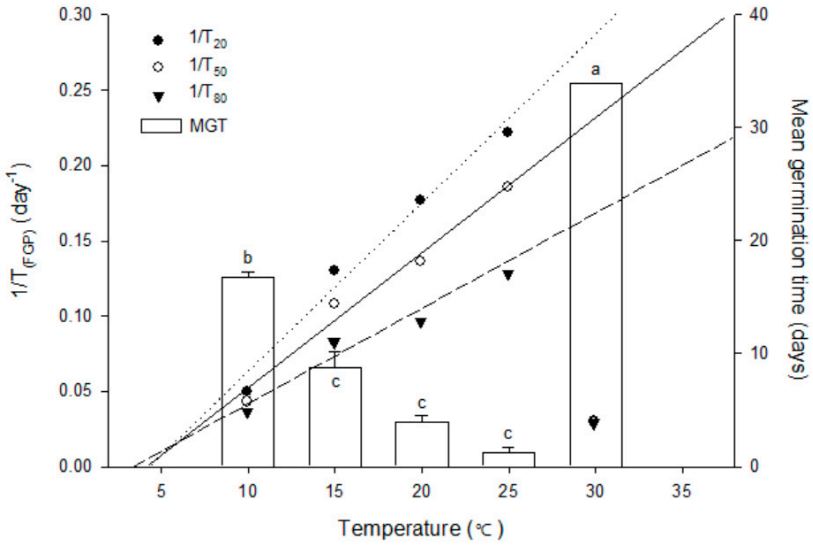
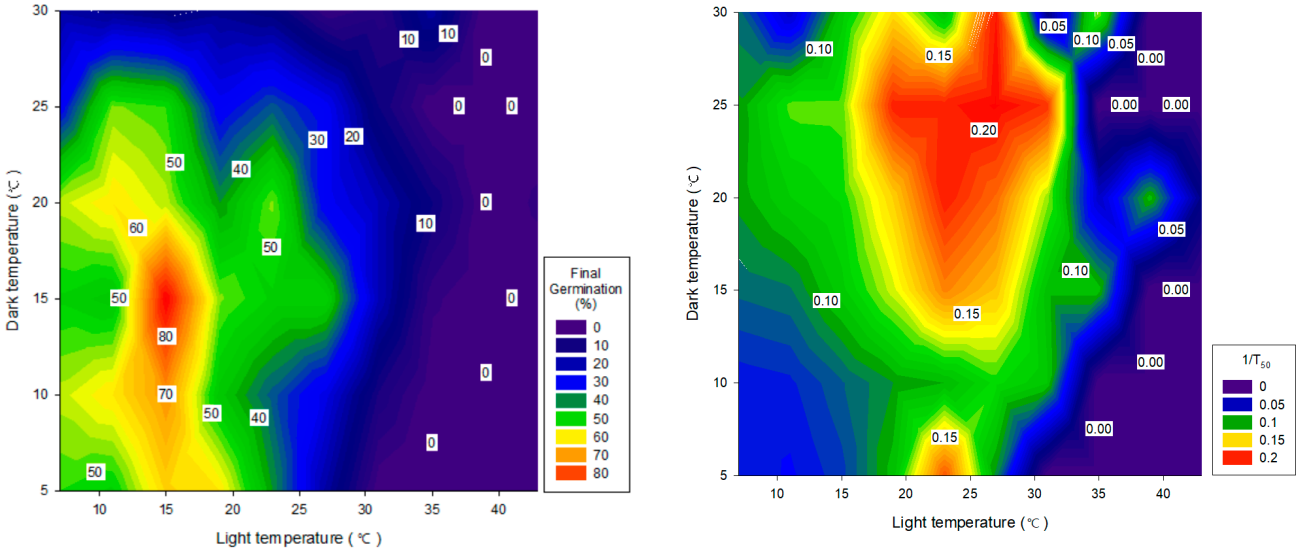


Figure 3. Effect of constant temperature regimes (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C) on the inverse of the time required to reach the FGP ($1/T_{(FGP)}$) and mean germination time, revealing linear relationships between $1/T_{(FGP)}$ and temperature in the sub-optimal range. The dotted line, solid line, and short dashed line indicate the linear regression for $1/T_{20}$, $1/T_{50}$, and $1/T_{80}$, respectively, passing through the base temperature (T_b). The T_b values were 4.56 °C, 4.68 °C, and 2.69 °C for $1/T_{20}$, $1/T_{50}$, and $1/T_{80}$, respectively. Vertical bars indicate standard errors (n = 4). Same lowercase letters indicate no significant difference ($p < 0.001$).

3.4. Alternating Temperature Regimes

To verify whether constant temperature regimes affect germination, a TGP was used to conduct different temperature experiments, with 58 alternating and constant (15 °C) temperature conditions. The highest FGP (86.6%) was observed at 15 °C in the temperature regime (Figure 4a), with a T_{50} value of 9–10 d ($1/T_{50}$: 0.10–0.11 d^{-1}) (Figure 4b). Most seeds germinated up to 50% until the temperature reached 23 °C, but the FGP markedly

decreased above 27 °C. Furthermore, the greater the temperature difference between light (day) and dark (night) periods, the lower the FGP (Figure 5).



(**a**) (**b**)

Figure 4. Germination response revealed by the analysis of FGP and the inverse of the number of days required to reach 50% of the FGP ($1/T_{50}$) of the seeds exposed to alternating temperature regimes using a thermal gradient plate: (**a**) FGP (%) at different temperatures; (**b**) $1/T_{50}$ at different temperatures ranging from 5 °C to 43 °C.

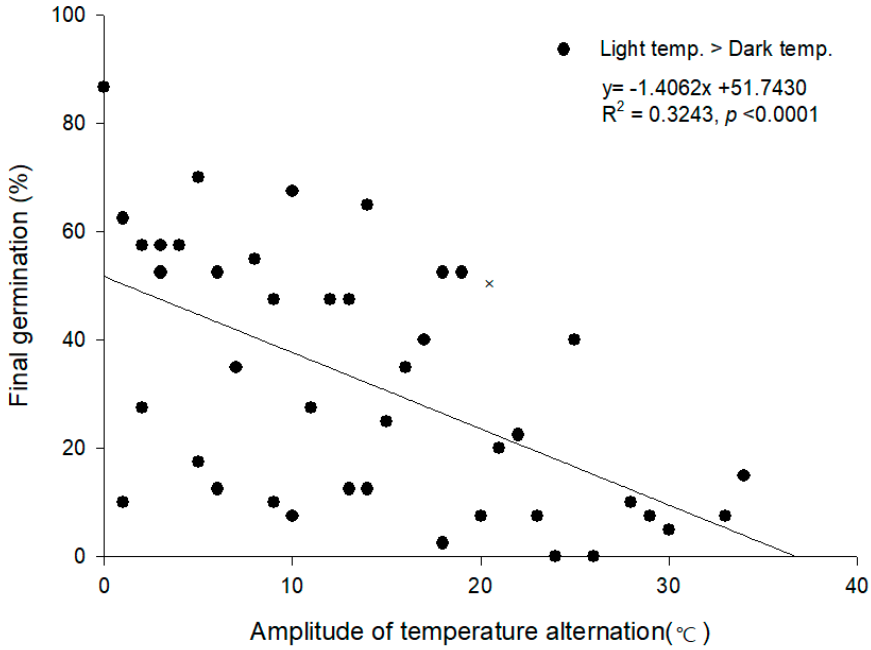


Figure 5. Relationship between the amplitude of temperature alternation (°C) and FGP (%) determined using the thermal gradient plate, with the temperatures during the light period being higher than those during the dark period.

4. Discussion

4.1. Seed Morphology and Characteristics

P. pusanensis, a rare species due to its small population, is classified as a protected species. It grows lying on the ground at short heights and has light blue flowers in racemes. The flowers at the end of the inflorescences stick together. The seeds in this study were collected one month after the inflorescences bloomed and all ovaries turned

from green to brown. At this time, some seeds were released from the basal flowers of an inflorescence. The shape of the seeds was as flat and round as a coin, and their average size was 1.05 × 0.96 mm (L × W). The E:S ratio during dispersal was 0.44. If the E:S ratio is 0.5 or less, the dormancy type is morphological dormancy (MD) and MPD [30]. Song et al. [10] found that the average length of *V. pusanensis* seeds collected from Pocheon on 23 September 2016 was 1.14 mm. Additionally, the seeds were of dwarf type (size: 0.3–2.0 mm) with an axile embryo [31]. Physiological dormancy in the embryo or tissue surrounding the embryo, which secretes a growth inhibitory agent to inhibit germination, was not observed, and because the E:S ratio increased, the dormancy was judged as morphological dormancy. The analysis results of the internal and external morphology showed that there was no visible structural difference, but the time of seed collection and the environmental conditions of the collection area (climate, precipitation, etc.) contributed to seed formation. Further, *P. pusanensis* had a high germination percentage at 15 °C due to the effect of breaking MD. During seed production, the environment has a significant impact on the behavior of progeny seeds [32]. The environmental conditions (temperature) near the parent tree can affect the germination percentage of their offspring, and can even affect later generations [33,34]. This has been demonstrated in wild and cultivated species. Further, the temperature difference in the parental environment can affect the degree of dormancy and germination behavior [35]. In addition to temperature changes, the altitude and season have been found to affect seed dormancy and offspring seed characteristics [32].

In this study, the MC of dried *P. pusanensis* seeds after collection was 4.32%, and the 1000 seed weight was 0.1188 g; these parameters were used to determine the number of seeds to be stored in the seed bank. MC is the most important factor responsible for maintaining seed viability in seed banks, and plays critical roles in determining the seed longevity [36]. Even small changes in the MC have remarkable effects on the shelf life of seeds [37]. Orthodox seeds are suitable for long-term storage if they have an MC of less than 8% at a 15% RH [38]. Furthermore, seeds stored at 4–5% MC are unaffected by seed storage fungi [39]. According to the present study, the MC of *P. pusanensis* seeds was less than 8%, which is suitable for long-term storage. According to the Kew seed information database [30], the storage behavior of 76 species of the *Veronica* genus was revealed. Among the 76 species, 98.68% were orthodox seeds, which could be stored in the seed bank for more than one year, while the storage of the remaining 1.32% was uncertain. Therefore, *P. pusanensis* seeds were considered orthodox.

In this study, for the first time, the fat and protein contents of *P. pusanensis* seeds were reported. The Kew seed information database has not reported any information about *P. pusanensis* in the genera *Veronica* and *Pseudolysimachion*. In the genus *Veronica*, 116 species were investigated for their storage behavior, mean 1000-seed weight, germination information, and oil and protein content. In the genus *Pseudolysimachion*, two species were investigated for their storage behavior and germination information. Of these, only three had information on their total crude fat and protein contents. The species with the highest total crude fat content were *Veronica spicata* L. (31.0%), *Veronica longifolia* L. (18.5%), and *Veronica salina* Schur (15.8%). The total crude protein content decreased in the following order: *Veronica longifolia* L. (27.5%) > *Veronica spicata* L. (26.2%) > *Veronica salina* Schur (21.4%). The total crude fat content of *P. pusanensis* was intermediate (22.4%), while its total crude protein content was the lowest, at 15.9%.

Information of seed characteristics is essential for the germination of stored seeds and is necessary to manage seed banks. This is because storage lipids are metabolized in seeds that require high energy for germination [40]. Furthermore, seed storage proteins are synthesized to serve as sources of carbon, nitrogen, and sulfur for the next generation of plants [41]. Additionally, the components of a seed (proteins, lipids, and starch) are related to seed longevity. When fat is oxidized due to aging during storage [42], the total crude fat content varies, thereby reducing the seed viability Therefore, to continuously maintain the viability of *P. pusanensis* seeds in the seed bank, seed characteristics that affect the lifespan and germination of seeds must be continuously monitored; additionally, the renewal cycle

should be determined to maintain viability. Seeds stored in the seed bank will lose their viability and longevity during storage. Since the longevity of seeds and the time it takes for the viability to decrease by 50% differ for each species, it is necessary to establish a renewal cycle for each species so that seeds in a seed bank can be efficiently managed. We are currently conducting follow-up studies to reveal the lifespan and renewal cycle for the species conservation of *P. pusanensis*.

4.2. Temperature Regimes

During condition 7 of the constant temperature experiment (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C), the seeds showed 94.38% of FGP at 15 °C, suggesting that the appropriate germination temperature for the seeds of *P. pusanensis* was 15 °C. In addition, it is considered to promote germination by breaking morphological dormancy. According to a previous study, *V. pusanensis* seeds showed >80% FGP in the temperature range of 15–25 °C. Comparing the germination response, *P. pusanensis* seeds exhibited a similar trend in GP, which increased with a certain increase in temperature and then decreased. However, at 25 °C, the temperature reactions were different from the previous study. Further, at 15 °C, 20 °C, and 25 °C, *P. pusanensis* showed a FGP of 94.38%, 74.44%, and 37.5% and MGT of 12.8 d, 3.9 d, and 1.2 d, respectively. However, previous studies did not report the exact value, and the range was inferred graphically [10]. At 15 °C, 20 °C, and 25 °C, the GP was reported to be 80–94.0%, more than 94.0%, and more than 94.0%, and the MGT was less than 10 d, 4.6 d, and 4.8 d, respectively. Although the two studies differed in the number of collection areas and the type of medium, it can be inferred that the germination response, such as germination (%) and germination rate (MGT, T_{50}), was observed at 15 °C in both studies. Further, there was a difference in the treatment at 20 °C and 25 °C, and, in particular, the opposite response was shown at 25 °C. Based on the results of the constant temperature experiment in the two studies, the most suitable temperature for breaking morphological dormancy and germination was judged to be 15–20 °C. These differences may be associated with differences in the GP across sampling areas. The percentage of seed germination for a species has been reported to be associated with specific environmental characteristics, including the total amount of precipitation and the variation in annual precipitation in the distributional range of the species [43–45]. *Heteropappus arenarius* Kitam., a species native to Korea, collected from different latitudes and longitudes, exhibited a different GP with temperature [45]. A Korean endemic species, *Abies koreana* Wilson, exhibited significantly different germination percentages and rates among populations [46].

Differences in the FGP of *P. pusanensis* may be related because of different distribution areas, and its indeterminate inflorescence. *P. pusanensis* seeds used for this study were collected from ovaries, except the basal flowers of the inflorescence of the mother plants. Furthermore, seeds produced at different heights of the parent plant exhibit differences in the level of dormancy and seed size, resulting in differences in dormancy breaking and germination requirements [47,48]. Seeds harvested from inflorescences at three different positions at the same time revealed that those collected from the bottom of the inflorescence (first seeds) exhibited a higher GP and fewer abnormalities than those collected from the second and third positions, which may exhibit different germination characteristics [49]. *Chelidonium majus* subsp. *asiaticum* Hara and 39 species have been reported to exhibit different germination characteristics depending on their location [18].

During condition 59 of the temperature experiment using TGP, the dormancy break and amplitude of temperature difference were negatively correlated. In the alternate temperature experiment, the lower the amplitude of temperature, the greater the dormancy breaks and the higher the possibility of germination (60.0%). Among the 59 conditions, in 11 conditions, *P. pusanensis* seeds had an FGP of 0–10%, and the night temperature had no effect.

5. Conclusions

In summary, according to the two temperature regime results, 15–20 °C was redefined as the optimum germination temperature range for *P. pusanensis* seeds, and it can be concluded that the smaller the amplitude of temperature alternation, the greater the morphological dormancy break, thereby increasing the possibility of germination. Additionally, theoretical conclusions were acquired on the basic characteristics and germination of *P. pusanensis* seeds. These results for *P. pusanensis* seeds stored in a seed bank provide useful data for ecological restoration and can also be applied for storage management. The findings will also be significant for the native plant horticulture industries.

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